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Application for Grant of U.S. Letters Patent

10 TITLE: NOVEL PHARMACEUTICAL COMPOSITIONS FOR TREATING AND SAVING AND
THE METHOD FOR THE PREPARATION THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to a novel pharmaceutical composition
15 for treating and saving the wounded, and to a method for the preparation
thereof.

[0002] Blood transfusion and fluid infusion are important measures to treat
and save wounded persons, especially persons suffering from traumatic shock.
Usually, the principle of transfusion is "to infuse components the patient is
20 deficient in, to supply how much the patient needs". For example, when the patient
has mainly lost blood, he should be transfused with blood, even though sometimes the patient needs to be transfused with blood from normal
individuals. When the patient has mainly lost plasma, plasma or plasma
volume expander should be transfused to him. When the patient has mainly
25 lost intercellular fluid, physiological saline should be infused. In fact, the
treating and saving measures of formulating physiological solutions on the
basis of normal body composition, or transfusing with blood from normal
individuals to the patient with evident physiopathological changes is to treat

the organism as a mechanical device, therefore these measures often have the following disadvantages:

[0003] (1) Blood transfusion: In general, the volume of blood transfused comes close to or exceeds the volume of blood lost. If a big amount of
5 blood is required, the blood source will be difficult to obtain, the cost will be expensive, and its preparation and storage require certain conditions. In addition, before transfusion, some time should be taken for blood typing and cross match tests, and only the substitutes could be used for the individuals with rare blood types. Blood transfusion could result in production of
10 anti-platelet antibodies and anti-leukocyte antibodies, as well as various hematogenic infectious diseases, for example AIDS, hepatitis B, hepatitis C, etc.

[0004] (2) Albumin infusion: There is a great demand, a great expense, difficulty in obtaining a source, complicated preparation method, and certain
15 requirements for the method of storage of albumin. After albumin infusion, it could effuse through capillaries, and couldn't be reabsorbed into vessel. Therefore interstitial edema will occur, and might result in pulmonary edema, renal failure, and cardiac insufficiency, thus enhancing the possibility for mortality. Albumin infusion could result in evident decrease of α_1 , α_2 , β ,
20 γ -globulins and fibrinogen, cause reduction of immunity, and affect function of blood coagulation.

[0005] (3) Infusion of fluorocarbon as plasma substitute: Such infusion requires a large amount of the substitute and simultaneous inhalation of oxygen with high component pressure. Otherwise the demand of the
25 organism is not met. The fluorocarbon as plasma substitute should be preserved at a low temperature, and its transport is not convenient. For example, thirty minutes before infusion, the patient should be injected with

10 mg of dexamethasone. The frozen injection for infusion should be thawed. Thus, the procedures are complicated. After infusion, observation should be continued for 5 – 10 min. The infusion of fluorocarbon as plasma substitute could induce adverse reactions, such as anaphylaxis, hypotension, 5 thrombocytopenia, hepatosplenomegaly, reduction of immunity, and abnormality of fibrinolysis system.

[0006] (4) Infusion of balanced buffers. The amount of balanced buffers infused should be three times more than the volume of blood lost to maintain the blood pressure. Sixty to eighty percent (60-80%) of the solution infused 10 could effuse out of the blood vessel, which results in tissue edema (for example, cerebral edema or pulmonary edema) and renal insufficiency, which in turn could cause difficulty for sequential therapy.

[0007] (5) Infusion of physiological saline. The amount of physiological saline infused should also be three times more than the volume of blood lost. 15 Its efficacy is poorer than balanced buffers, and its adverse effects are more significant.

[0008] To solve the problems in blood transfusion and infusion, the skilled in the art had studied anti-shock therapy with hypertonic sodium chloride solution. For example, 7.5% (w/v) NaCl solution as suggested by Velasco. 20 The hypertonic NaCl solution, however, has some toxicity to the organism.

[0009] Most investigators have proposed intravenous injection of hypertonic NaCl solution for anti-shock therapy, but it usually leads to obvious complications, such as hypotension, rupture of blood cells induced by extra hypertonic solution, cardiac insufficiency, decreased renal function and 25 disorders of the nervous system.

[0010] Thus, it can be shown that there is a demand for novel anti-shock drugs to reverse the physiopathological condition of shock, in order to obtain

time for sequential therapy after the emergency treatment, and to create opportunity for improved effect of treating and saving the wounded and patients, and increasing survival rate.

5 [0011] An object of the present invention is to provide a pharmaceutical composition with a convenient source, that requires less dosage, and that has rapid and better efficacy, less side effects, wider uses, is not restricted by blood type and does not require special storage conditions.

[0012] Another object of the present invention is to provide a method for the preparation of the said pharmaceutical composition.

10 [0013] The present invention proposes a new concept of liquid therapy for shock, based on three aspects of thinking. The first aspect relates to the present unreasonable dosage regimen, and adopts the following principle: “to infuse what component the patient needs, then to infuse how much the patient needs”. Based on the physiopathological status of the patient with
15 shock, there is a prior demand for the compound solution containing hypertonic sodium ion (or a combination of various crystals or a combination of various crystals and various colloids, etc.) to preliminarily improve micro-circulation, tissue perfusion, and hemodynamics immediately. Then in the light of practical demand the isosmotic solution or proper hypoosmotic
20 solution or whole blood or concentrated red blood cell suspension is administered, in order to permit the latter infused solution better action when the patient’s condition has improved preliminarily by the earlier hypertonic solution, and to remit over-dehydration of some cells caused possibly by the hypertonic solution infused earlier. The second aspect relates to the present
25 unreasonable ratio of colloids and crystals in the transfusion for patient with shock when prepared on the basis of their normal physiological concentrations. In view of the fact that the property and volume-expanding

ability of artificial colloids are different from albumin in blood, it is considered that the transfusion with suitable ratio of colloids and crystals should be administered on basis of the physiopathological status of patients, rather than the normal physiological proportion. Thus medicine 5 administration according to indications could reduce the dosage, increase the efficacy, and decrease the complications. The third aspect relates to inadequacy in the present anti-shock experimental studies (such as animal model, reasonable concentration and infusion rate of hyperosmotic solutions), a great number of experimental studies have been conducted, and met with 10 success.

[0014] Based on the above three aspects of thinking from the theoretical researches and the clinical experiences, the particular embodiments of the present invention have been completed through animal experiments and clinical practice.

15 [0015] The present invention is achieved through the following embodiments. A pharmaceutical composition comprising 1.5-6.9% (w/v) of one or more substances selected from sodium chloride, sodium bicarbonate, potassium chloride, magnesium sulfate, calcium chloride, calcium gluconate, calcium lactate, sodium lactate, sodium acetate and Tris (Hydroxy methyl) aminomethane; and 3-18% (w/v) of one or more substances selected form 20 hydroxyethyl starch, dextran, carboxy methylstarch, polyvinyl-pyrrolidone (PVP), gelatin derivatives, condensed glucose, glucose, fructose, lactose, glycerin, xylitol, sodium alginate, N-2-hydroxypropylacrylamide, ethylene epoxide-polypropylene glycol, pectin, mannitol, and penta hydroxyethyl 25 starch (Pentastarch) as well as the remainder of conventional injections, as long as sodium chloride is not less than 1.5% (w/v), and the concentration of sodium ion is not more than that of 6.9% (w/v) sodium chloride solution or

equivalent.

[0016] The preferred composition of the present invention consisting of 4.2±0.2 g. sodium chloride and 7.6±0.6 g. hydroxyethyl starch per 100 ml.

[0017] In the composition, the said hydroxyethylstarch contains at least 10%
5 hydroxyethylstarch with molecular weight of 25,000-45,000 atomic mass
units (amu).

[0018] The said dextran has molecular weight of 40,000-230,000 amu,
carboxymethyl starch has molecular weight of 30,000-80,000 amu, PVP has
molecular weight of 5,000-700,000 amu, condensed glucose has molecular
10 weight of 8,000-12,000 amu; sodium alginate has molecular weight of
20,000-26,000 amu, pectin has molecular weight of 20,000-40,000 amu, and
pentahydroxyethyl starch (the product of DuPont Company (Pentastarch)) has
molecular weight of 264,000 amu.

[0019] The said gelatin derivatives have molecular weight of 20,000-35,000
15 amu and are selected from urea, conjugated gelatin, modified liquid gelatin,
oxidized polygelatin and degraded gelatin poly-peptide.

[0020] Conventional injections are selected from water for injection,
physiological saline, balanced buffers, glucose solution, sodium lactate
solution, sodium acetate solution, Tris solution, glucose solution and sodium
20 chloride solution.

[0021] The composition of the present invention is prepared according to the
following procedure: dissolving 3-18 g. of total amount of one or more
substances selected from hydroxyethyl starch, dextran, carboxymethyl starch,
PVP, gelatin derivatives, condensed glucose, glucose, fructose, lactose,
25 glycerin, xylitol, sodium alginate, N-2-hydroxypropylacrylamide, ethylene
epoxide, polypropylene glycol, pectin, and pentahydroxyethyl starch in 100
ml of total volume of one injection or mixture of several injections selected

from water for injection, physiological saline, balanced buffers, glucose solution, sodium lactate solution, sodium acetate solution, Tris solution, glucose solution and sodium chloride solution; then adding 1.5 g. sodium chloride and 0-5.4 g. of one or more substances selected from sodium 5 chloride, sodium bicarbonate, potassium chloride, magnesium sulfate, calcium chloride, calcium gluconate, calcium lactate, sodium lactate, sodium acetate, and Tris; with the proportion described above, then mixing, and dissolving, to obtain the composition of the present invention.

[0022] The preferred technical embodiments are as follows: (i) preparation 10 of hydroxyethyl starch: According to the proportion of 1:0.8-0.875:0.04-0.042 (w/v/v) corn starch or sorghum starch, 95% ethanol, and 35-38% hydrochloric acid are mixed, the temperature is raised to 65-80 °C for hydrolysis of starch, 16% sodium hydroxide solution is added in proportion of 0.6-0.7:1 (v/w) of stated solution versus starch. Then ethylene 15 epoxide is added in proportion of 0. 35-0. 5:1 (w/w) of ethylene epoxide versus starch, then the mixture is heated to 65-75 °C to cause starch hydroxyethylation. (ii) Formulation of the composition: Appropriate volume of water is added, to prepare $7.6 \pm 0.6\%$ (w/v) hydroxyethylstarch solution. Suitable amount of activated charcoal is added to discolor the 20 solution through adsorption. After filtration, the pH is adjusted to 5.5-7, and the total of 4 ± 0.2 g. of sodium chloride is added. A suitable amount of activated charcoal is added again for adsorption and discolor action. After filtration through $0.8\mu\text{m}$ micro-porous filter, the preferred composition of the present invention is obtained.

25 [0023] The present invention is further illustrated in detail by the following examples.

Preparative example.

Preparation of hydroxyethyl starch.

[0024] 100 g. corn or sorghum starch are mixed with 87 ml. of 95% ethanol and 4.2 ml. of 35% hydrochloric acid. The temperature is raised to 70°C to 5 hydrolyze starch then 60 ml. of 16% sodium hydroxide solution is added, then 45 g. epoxyethane is added, and the mixture is heated to 70°C to complete hydroxyethylation of starch. According to the formula and preparative method described above, hydroxyethyl starch with molecular weight of 25,000-45,000 amu is obtained.

10

[0025] Example 1.

Prepare according to the following proportion:

hydroxyethylstarch	7.6 g.
15 sodium chloride	4.2 g.
water for in injection	added to 100 ml.

[0026] 7.6 g. hydroxyethyl starch are dissolved in 100 ml. of water for injection. One half gram (0.5 g.) of activated charcoal is added, and the mixture is heated at 90°C for 15 min under stirring. After filtration through 20 an asbestos plate filter, 4.2 g sodium chloride (purity pharmaceuticals use) are added, and dissolved with stirring. 0.5 g. activated charcoal is added, and the mixture is heated at 90°C for 10 min. under stirring. After filtration through an asbestos plate filter and 0.8μm micro-porous filter, the resultant filtrate is transferred into 250 ml. or 500-ml. glass or plastic bottles (bags).
25 After sealing, the bottles or bags are 1.05 kg/cm² and 121-123 °C for 15-30 min. for sterilization, to obtain the pharmaceutical composition of the present

invention.

[0027] Example 2.

Prepare according to the following proportion:

5	dextran	9 g.
	hydroxyethyl starch	3 g.
	sodium chloride	1.5 g.
	sodium bicarbonate	3.4 g.
	physiological saline	added to 100 ml.

- 10 **[0028]** The above-mentioned dextran (produced by Shanghai Glucose Factory), hydroxyethyl starch (prepared according to preparative example) are dissolved in physiological saline, and adsorbed and discolored with activated charcoal according to the method stated in Example 1. Then the above-mentioned sodium chloride, sodium bicarbonate, are added in turn, and
15 dissolved with stirring. Thereafter, the obtained solution is discolored, filtered, sterilized and filled, to obtain the pharmaceutical composition of the present invention.

[0029] Example 3.

20 Prepare according to the following proportion:

	polyvinyl-pyrrolidone (PVP) (produced by Bayer)	12 g.
	sodium chloride	2 g.
	sodium acetate	4 g.
	10% glucose solution	added to 100 ml.

- 25 **[0030]** According to the method described in Example 2, except that dextran and hydroxyethyl starch is replaced by PVP, sodium bicarbonate is replaced with sodium acetate, and physiological saline is replaced with glucose solution, the composition of the present invention is obtained.

dextran 9 g.

[0031] Example 4.

Prepare according to the following proportion:

5 sodium alginate (produced by Nanning 18 g.

Pharmaceutical Factory, Guangxi)

sodium chloride 1.5 g.

water for injection

1.5 g.

water for injection. added to 100 ml.

[0032] According to the method described in Example 1 to prepare the
10 above-mentioned formulation, thus obtaining the pharmaceutical composition
of the present invention.

[0033] Example 5.

Prepare according to the following proportion:

15 pectin (produced by PLA No. 185 Hospital) 3 g.

Pentahydroxyethylstarch (produced by 4 g

DuPont Company)

4 g.

mannitol

7 g.

20 2% sodium lactate lution added to 100 ml.

[0034] According to the method in described in Example 1, pectin Pentahydroethyl starch and mannitol are dissolved in sodium lactate solution, then sodium chloride is added and dissolved.

25 [0035] Example 6.

Prepare according to the following proportion:

condensed glucose (produced by southwest No. 5 Pharmaceutical Factory Chongqing) 7 g.
N-2-hydroxy propyl acrylamide 2 g.
sodium chloride 4.4 g.
5 water for injection added to 100 ml.

[0036] Using the method in Example 1, the pharmaceutical composition of the present invention is prepared according to the above mentioned formula.

[0037] Example 7.

10 Prepare according to the following proportion:

fructose (produced by Shanghai No. 2 Reagent Factory) 5 g.
xylitol (produced by Liaoyang organic Chemical Plant) 4 g.
sodium chloride 4.8 g.
water for injection added to 100 ml.

[0038] Using the method in Example 1, the pharmaceutical composition of the present invention is prepared according to the above-mentioned formula.

20 [0039] Example 8.

Prepare according to the following proportion:

glycerin 2 g.
lactose (produced by Shanghai No. 2 chemistry Reagent Factory) 5 g.
sodium chloride 6 g.
water for injection added to 100 ml.

[0040] Using the method in Example 1, the pharmaceutical composition of the present invention is prepared according to the above-mentioned formula.

[0041] Test 1: Animal Experiment

From adult healthy hybrid dogs, regardless of sex, under local anesthesia, isolate femoral artery and femoral vein, then insert catheters
5 respectively.

[0042] The arterial duct is connected to a CF-II model monitor of cardiovascular function [Shanghai approval document number: Hu-Yao-Qi-Jian (Zhun)-97-221103] to monitor cardiovascular status. The dogs are bled to monitor cardiovascular status. The dogs are bled to an
10 average arterial pressure (MAP) of 40-50 mmHg for a period of about 15 min. This blood pressure level is maintained for 1 hour, then the product prepared in Example 1 is infused at the dose of 8 ml/kg.

[0043] The cardiovascular function and urine volume is monitored over 4 hours after infusion. In the following tables, the blood pressure and other
15 indexes are expressed as percentage of their basal levels respectively, the unit of urine volume is ml/kg body weight/h.

Table 1
**Comparison between the composition of the invention and whole blood in equal volume of recovery of
Cardiovascular function in dogs with shock. [unit: % compared with respective basal level]**

		after transfusion			
		30 min	1h	2h	3h
Systolic pressure	Composition of the invention equal volume of whole blood	75±4 (p<0.01)	76±4 (p<0.01)	77±4 (p<0.05)	78±5 (p<0.05)
diastolic pressure	Composition of the invention equal volume of whole blood	67±5 (p<0.01)	69±6 (p<0.01)	73±4 (p<0.05)	73±4 (p<0.05)
average arterial pressure	Composition of the invention equal volume of whole blood	77±5 (p<0.01)	79±5 (p<0.05)	81±4 (p<0.05)	81±5 (p<0.05)
cardiac contractility	Composition of the invention equal volume of whole blood	69±6 (p<0.01)	73±8 (p<0.01)	76±7 (p<0.05)	76±7 (p<0.05)
cardiac output	Composition of the invention equal volume of whole blood	105±13 (p<0.05)	106±14 (p<0.01)	103±14 (p<0.01)	106±12 (p<0.01)
end-diastolic volume	Composition of the invention equal volume of whole blood	82±4 (p<0.05)	82±3 (p<0.05)	83±4 (p<0.05)	84±3 (p<0.05)

Table 2

Comparison between composition of the invention and equal volume of whole blood
For urine volume in restoration stage in shocked dogs [unit: ml/kg/h]

	after transfusion			
	1h	2h	3h	4h
Composition of the invention	2.23±1.03	0.94±0.22	0.95±0.29	1.00±0.30
equal volume of whole blood	0.33±0.21	0.27±0.16	0.73±0.41	0.61±0.25
	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.05)

[0044] The composition of Example 1 in accordance with the invention was administered to 48 patients in Hefei No. 105 Hospital, Anhui province. The total effective rate was 100%. Most patients had the blood pressure raised, urine volume increased, and the limbs became warm during transfusion. In several patients whom conventional drugs couldn't reverse, the composition of the Example 1 begin to play its role 5-10 minutes after infusion. The circulatory function of patients recovered basically, and there were no obvious clinical complications.

[0045] Test 3. Experiment of acute toxicity

Dogs given 2.5 times the dosage for humans showed no adverse effects. At 5 times the recommended dosage, salivation and vomiting were seen in the dogs. At 3.75 times the recommended dosage vomiting was seen without salivation in the dogs. All the above administered dogs survived more than 45 days. At 7.5 times the recommended dosage death occurred in the administered dogs and focal hemorrhage was seen in the lungs as target organs.

[0046] The composition of the present invention could be infused through the veins at a dose of 8 ml of the composition of the present invention per kg body weight. It could be used directly in treating and saving patients with shock, combined injuries or hematorrhea, etc in order to reverse the physio-pathological status of patients and to obtain time for sequential treatment.

[0047] As compared with the prior art, the pharmaceutical composition of this invention has the following prominent features and improvement:

[0048] 1. Greatly decreased volume of transfusion: In general, the dose for most patients is 500 ml. or less. Even if the patients suffered from lethal hematorrhea, to infuse only 1/4 to 1/6 of volume of lost blood is enough.

Thus, it could obviously decrease the incidence rate of tissue edema or overload of the heart.

[0049] 2. Rapid curative effect just during 5-10 minutes after infusion, the hemodynamics has been improved significantly.

[0050] 3. Good efficacy. As Test 1 showed, the composition of the invention had better efficacy than that of equal volume of fresh whole blood. Moreover, although the composition of the invention has no oxygen-carrying action, it could improve micro-circulation and general status to decrease oxygen consumption and to increase oxygen transport. Thus, at least 50% of blood transfused could be saved, which could mitigate the contradiction with short supply of blood, decrease the complications induced by blood transfusion, and reduce the economic burden for patients.

[0051] 4. Maintain once of efficacy for long time. As Test 1 showed, after infusion of the composition of the invention, the improvement of hemodynamics and general condition could be maintained more than 3-4 hours, even if all other infusion and drugs were not administered.

[0052] 5. Unnecessary special condition for storage: The composition could be stored at room temperature, simply used infused intravenously or intraosseously and conveniently transported, without special devices and special vehicles.

[0053] 6. Unnecessary blood typing and cross match tests: It is suitable for individuals with any blood type. Thus, valuable time could be gained to rescue the wounded and patients.

[0054] 7. Wider uses: It could widely be used in the treatment of patients with shock of various types, brain trauma, burn, combined injuries, cardiogenic shock induced by myocardial infarction of right ventricle, hypotension induced by hemodialysis, biliary pancreatitis, cardiovascular

intoxication induced by narcotic, hepatic echinococcosis, and patients under operation.

[0055] 8. Change of administration model: The composition of the invention could be infused drop by drop intravenously, instead of pushing so it could be conveniently used with less complications.

[0056] In general, as compared with prior therapy, the composition of the present invention possesses unique benefit and innovation, for treating and saving the wounded and patients.